

Development of Antimicrobial Coatings for Improving the Microbiological Safety and Quality of Shell Eggs[†]

TONY Z. JIN,^{1*} JOSHUA B. GURTLE¹, AND SI-QUAN LI²

¹U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 190382; and ²Michael Foods Inc., 120 Tower Street South, Gaylord, Minnesota 55334, USA

MS 12-460: Received 15 October 2012/Accepted 19 December 2012

ABSTRACT

This study was conducted to develop antimicrobial coatings to decontaminate and prevent cross-contamination of shell eggs. Egg shells were inoculated with nalidixic acid-resistant *Salmonella enterica* Enteritidis strains OB030832, OB040159, and C405 and treated with antimicrobial coatings. Polylactic acid served as a nonedible polymer, and chitosan served as an edible polymer carrier of natural antimicrobials, including nisin, allyl isothiocyanate (AIT), lauric arginate ester (LAE), and organic acids. Increases of AIT concentrations or addition of nisin to AIT in either the polylactic acid or chitosan coating solutions resulted in greater reductions of *Salmonella*. Chitosan coatings with 0.1, 0.5, and 1.0% LAE reduced *Salmonella* by 1.7, 2.5, and 5.2 log CFU/cm², respectively. Shell eggs treated with 1.0 and 0.5% LAE in chitosan coatings had nondetectable *Salmonella* cells (<0.5 log CFU/cm²) after 3 and 7 days of storage at 7°C, respectively, and no outgrowth was observed up to 28 days. Coating treatments significantly reduced weight loss of shell eggs during 12 weeks of storage at 7 or 4°C. This study demonstrates an alternative and effective intervention technology for decontaminating shell eggs and provides an alternative approach to reduce possible recalls and outbreaks associated with pathogen contamination on shell eggs and in egg products.

The United States produced 6.38 billion dozen eggs in 2008. Of these, approximately 59% went to retail, 32% went to further processing, 9% went to food service, and 0.7% were exported (1). The egg industry adds \$4 billion annually to the U.S. economy. From April 2009 to June 2010, the 30-day average egg consumption increased to 33 eggs per household, its highest level in 7 years (47).

Eggs and egg products are the single class of foods most frequently implicated in *Salmonella* outbreaks in Europe (17). Various *Salmonella enterica* serovars may be isolated from eggs, but the most common one is *Salmonella* Enteritidis (16, 17, 21, 24), which has been associated with most eggborne outbreaks in the past 3 decades. A multistate outbreak of human *Salmonella* Enteritidis infections associated with shell eggs occurred in 2010, with more than 1,000 victims, resulting in over 550 million shell eggs being recalled from the market (10). The *Salmonella* Enteritidis contamination of shell eggs continues, despite implementation of voluntary national *Salmonella* Enteritidis traceback procedures and intensified efforts to educate food handlers and enforce safe food handling practices. According to 2005 estimates, 20% of U.S. layer flocks are *Salmonella* Enteritidis positive and 1 in every 3,600 eggs is contaminated with *Salmonella*, resulting in 13.9 million

Salmonella Enteritidis-positive shell eggs per year (51). The Food Safety and Inspection Service has further concluded that this rate of contamination leads to approximately 130,000 illnesses and up to 139 deaths per year. More than 90% of cases of foodborne salmonellosis caused by *Salmonella* Enteritidis are attributed to contaminated shell eggs (45).

Salmonella can contaminate shell egg internal contents by (i) transovarian transmission, (ii) transoviductal transmission prior to shell calcification, and/or (iii) penetration through the shell pores, which is facilitated by a negative atmospheric temperature differential and the vacuum effect that draws bacteria into the egg during washing and/or storage. Contamination of the shell can occur in the cloacae or can be due to environmental contamination, which is an important source of this organism (31). Burow (6) reported that 0.42% of shells were contaminated with *Salmonella*, while the United Kingdom Public Health Laboratory Service isolated *Salmonella* Enteritidis in 1.07% of shells and 0.66% of egg containers (2). Telo et al. (49) reported a contamination rate of 1.26% when analyzing pools of five eggshells.

The presence of *Salmonella* on the shell obviously poses a risk to human health. Apart from possible penetration into the egg, contamination of contents may occur when the shell is broken, prior to processing, which can also lead to cross-contamination issues with other food products. If the contaminated product is consumed raw or insufficiently cooked, the bacterium can lead to illness.

* Author for correspondence. Tel: 215-836-6904; Fax: 215-233-6559; E-mail: tony.jin@ars.usda.gov.

† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

According to the Public Health Agency of Canada (43), the human oral infectious dose for *Salmonella* species is 10^2 to 10^3 CFU; nevertheless, some report this dose being as low as 10 (33) to 28 cells (53), while 10 to 20 CFU per egg has been considered a normal level of contamination (23).

Various methods have been proposed for decontaminating the surface of eggs, such as dry cleaning or washing with water, usually containing a sanitizing agent (e.g., sodium hypochlorite). Washing of shell eggs for retail sale is a matter of continuous debate. This method, followed by chilled storage, is a common practice with on-line systems in the United States, Canada, Australia, and Japan. The major disadvantage is the potential damage to the cuticle that may favor trans-shell contamination with bacteria and moisture loss (15). Further, most *Salmonella* Enteritidis outbreaks have generally involved grade A eggs that have been washed and disinfected and meet other requirements of the state for shell quality (48). The U.S. Department of Agriculture egg pasteurization standards, as recorded in the U.S. Code of Federal Regulations (9 CFR 590.570) (50), requires liquid egg white (albumen), liquid whole eggs, and egg yolk separated from shell eggs to be thermally treated minimally at 56.7, 60.0, and 61.1°C, respectively, for 3.5 min and requires egg white to be treated at 55.6°C and plain yolk at 60.0°C for 6.2 min to ensure egg safety against *Salmonella* and other foodborne pathogens. Nevertheless, many egg dishes served in restaurants and for specialty meals require the use of high-quality fresh shell eggs (e.g., over easy, poached, hard cooked, eggs Benedict, coddled, deviled, specialty egg dishes).

The current technique for in-shell pasteurization of eggs involves heating the eggs in a liquid medium at a specifically designed temperature for a specified time period, depending on the size of the eggs. Although the process is known to increase the Haugh value of eggs, in-shell pasteurization also may lead to overheating of egg white proteins, leading to some denaturation, coagulation, and loss of albumen transparency (22), which greatly affects the eggs' functional properties (46). Methods not requiring such thermal pasteurization treatments, therefore, may be a better alternative to reduce the overall potential microbial hazards of shell eggs.

A commercial process was recently developed for rapidly cooling shell eggs using cryogenic CO₂. The use of cryogenic cooling increased the overall Haugh Unit values and resulted in the cryogenically gas-cooled eggs maintaining an AA quality at least 1 week longer than their traditionally cooled counterparts. However, the use of carbon dioxide to cool shell eggs also resulted in an increased percentage of cracked eggs (32) and cannot achieve the desired microbial reduction, although it may prevent further growth of *Salmonella* within the treated eggs.

To overcome these problems, considerable attention has been given to the development of coating materials for preservation of eggs, including polysaccharides, proteins, or lipids, alone or in combination (12, 20, 35, 42, 55). Egg coatings delay the interior quality deterioration rate and improve the mechanical properties of the shell. Wong et al.

(54) reported that various coatings (viz., mineral oil, soy protein, wheat gluten, or corn zein) can enhance the mechanical properties of shell eggs and interior quality. Chitosan coatings have proven effective in preserving the interior quality of eggs. Herald et al. (20) studied the quality of eggs coated with a wheat gluten solution. Xie et al. (55) showed that soy protein isolate, whey protein isolate (WPI), and wheat gluten coatings can enhance the functional properties of shell eggs and minimize egg microbial contamination. Protecting egg surfaces with coatings (e.g., chitosan, WPI, and shellac) has improved sensory attributes and led to longer shelf life. The WPI coatings had the best consumer perception among the three materials tested due to the high gloss and transparency of WPI coatings. However, most of these studies emphasized egg quality, and very little information on pathogen reduction by the coating process is available, particularly to achieve a 5-log reduction while maintaining or improving quality.

Previous studies in our laboratory have demonstrated that antimicrobial films or coatings with U.S. Food and Drug Administration generally recognized as safe (GRAS) antimicrobials are effective in reducing pathogens in various foods (26, 27, 29, 30, 38, 39). Polylactic acid (PLA) bottle coatings with 500 µl of allyl isothiocyanate (AIT) completely inactivated 3 and 7 log CFU/ml *Salmonella* in egg albumen, after 7 and 21 days of storage at 10°C, respectively, while PLA coatings with 200 µl of AIT in combination with 250 mg of nisin reduced *Salmonella* populations to an undetectable level (<10 CFU/ml) after 21 days of storage (27). Gurtler et al. (18) demonstrated that liquid whole egg containing AIT inactivated *Yersinia pestis*, a pathogen in the same family as *Salmonella* (*Enterobacteriaceae*), as determined by direct surface plating as well as selective enrichment. In another study, antimicrobial PLA coatings reduced populations of *Escherichia coli* O157:H7 and *Salmonella* Stanley on apples by up to 4 log CFU/cm² at 1 day and 4.7 log CFU/cm² at 14 days, compared with controls (29). Coating with chitosan and three acids reduced *Salmonella* by more than 6 log on tomato stem scars (28), and AIT in chitosan coatings reduced it by more than 5 log on cantaloupes (11). These results indicate that the use of polymers as carriers of antimicrobials not only provides controlled release of antimicrobials but also provides dramatic reductions in pathogen populations due to their affinity for food particles and inactivation by reactive food components. Our hypothesis, in the present study, was that similar antimicrobial coatings should also work for reducing pathogens on shell eggs. Therefore, the objectives of this study were to develop a simple and economic coating technology, which would (i) achieve a 5-log reduction of *Salmonella* on the surface of shell eggs and (ii) serve as a barrier to reduce weight loss of shell eggs to preserve egg quality and extend shelf life.

MATERIALS AND METHODS

Materials. Grade A shell eggs (average weight, 55 ± 2 g) were purchased at a local grocery store. Shell eggs without visible cracks were washed in tap water to remove debris and surface sanitized with 70% ethanol to remove background bacteria present

on the surface. Shell eggs were stored at 4°C and brought to ambient temperature (22°C) prior to use. Chitosan (150 kDa, 75 to 85% deacetylation), AIT (95% purity), and nisin (2.5% purity) were purchased from Sigma Aldrich (St. Louis, MO). Lauric arginate ester (LAE) solution (CytoGuard) containing 20% LAE was from A&B Ingredients (Fairfield, NJ). Methylene chloride, food grade acetic acid, lactic acid, and levulinic acid were from Fisher Scientific (Fairlawn, NJ). PLA resin (4060D) was from Natureworks (Minnetonka, MN).

Bacterial cultures and inoculum preparation. A cocktail of *Salmonella* isolates resistant to nalidixic acid was used in this study. *Salmonella* Enteritidis OB030832, OB040159, and C405 (all egg isolates) were from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center.

One day prior to experiments, 100 µl of each of the above-mentioned strain cultures was transferred to each of three individual test tubes containing 10 ml of tryptic soy broth (TSB) with 100 ppm of nalidixic acid (TSBN). The inoculated TSBN tubes were incubated overnight at 37°C. Individual suspensions were centrifuged at $1,800 \times g$ for 10 min. Tubes were immediately removed from the rotor, and the supernatant fluid was decanted from each tube. Each tube was vortexed for 1 min to break up the pellet present at the bottom with the addition of 0.7 ml of 0.1% peptone water. The inoculum was prepared by combining 0.7 ml of each strain mixture into a single composite. Populations of the resulting composite inocula (ca. 10^8 CFU/ml) were determined by plating appropriate serial dilutions onto tryptic soy agar (Difco) plus 100 ppm of nalidixic acid plus 0.1% sodium pyruvate (Sigma Aldrich) (TSAPN) and incubating them for 24 h at 37°C.

Inoculation of shell egg. A spot inoculation method was used in this study. One hundred microliters of a three-strain inoculum was spotted onto correspondingly marked egg shells (6.45 cm²). The inoculum was applied in approximately equal volumes at 10 locations over the marked surface to facilitate drying. Shell eggs were air dried at 22°C for 2 h in a laminar flow biosafety hood to permit cell attachment.

Coating solution preparation and coating treatment of shell eggs. Coating solutions were prepared as described in our previous studies (11, 27). Briefly, chitosan coating solutions included 200 mg of chitosan in 10 ml of an acid solution containing 2% each of acetic, lactic, and levulinic acids, while the PLA coating solution included 200 mg of PLA resin in 10 ml of methylene chloride. Nisin (250 mg), 200 or 600 µl of AIT, or 50 (0.1%), 250 (0.5%), and 500 (1.0%) µl of LAE were added into chitosan or PLA coating solutions. These mixtures were stirred with a magnetic stir bar on a stir plate until the polymers were completely dissolved. The coating solution (1 ml) was evenly distributed throughout the demarcated square areas of the shell egg with a small paint brush. The coated and noncoated (control) shell eggs were placed in a biosafety hood at room temperature (ca. 22°C) for 24 h prior to microbiological analyses. For storage tests, shell eggs were held in a temperature-controlled chamber at 7°C.

Microbiological analysis. At each sampling time, eggs were each aseptically cracked using the EZ-Cracker instrument, and contents were discarded. Each broken eggshell, with adhering membranes, was added to a sterile 50-ml centrifuge tube with 30 ml of sterile 0.1% peptone water and macerated with a sterile round glass rod and vortexed for 2 min. A 1-ml sample from each homogenate was serially diluted up to five times in 9 ml of 0.1%

peptone water. Next, 100 µl of each dilution was spread plated onto duplicate TSAPN plates. All plates were incubated at 37°C for 24 h, and CFUs were enumerated.

Water loss measurements. For water loss tests, whole eggs were coated, and five eggs from each respective coating were weighed at each sampling time over the 12-week study. Average weights were reported.

Statistical analysis. All experiments were conducted in triplicate using five eggs per treatment. Data were pooled and analyzed by analysis of variance with SAS version 9.1 software (SAS Institute, Cary, NC). Duncan's multiple range test was used to determine the significant differences of mean values. Significance was defined at *P* values of <0.05.

RESULTS

Effect of egg coatings containing AIT and nisin on inactivation of *Salmonella*. Two biopolymers (chitosan as an edible polymer and PLA as a nonedible polymer) and two natural antimicrobials (AIT and nisin), which had been used for our previous studies (Chen et al. (11), Jin and Gurtler (27, 28), and Jin (26)), were used in our first trials for shell eggs. Figure 1 shows the effects of PLA and chitosan coatings with AIT and nisin on reduction of *Salmonella* on egg shells. PLA coatings with 20 and 60 µl of AIT per ml reduced *Salmonella* by approximately 0.95 and 1.2 log CFU/cm², respectively, while chitosan coatings with 20 and 60 µl of AIT per ml reduced the organism populations by 1.1 and 1.7 log CFU/cm², respectively. The addition of nisin to the coating with AIT reduced *Salmonella* by approximately 2.5 log units for the PLA coating and 2.9 log units for the chitosan coating, which achieved significantly more reduction of *Salmonella* than the coating with AIT only. When the same amount of antimicrobials (AIT or nisin) was incorporated into the coatings, the chitosan coating showed more microbial reduction than did the PLA coating. The explanation for this phenomenon is that chitosan itself has antimicrobial activity while PLA does not, which is consistent with our previous publications (11, 30). Therefore, chitosan coatings with AIT were used for further comparison studies with chitosan plus LAE.

Effect of egg coatings containing LAE on inactivation of *Salmonella*. Figure 2 shows a comparison of chitosan coatings with AIT- and LAE-supplemented coatings. Increasing concentrations of LAE within the chitosan coatings led to concomitant increases in *Salmonella* inactivation on egg shells, while chitosan coatings with 0.1, 0.5, and 1.0% LAE reduced *Salmonella* by 0.5, 2.5, and 4.7 log CFU/cm², respectively. Adding 0.1% LAE in chitosan coatings did not significantly increase microbial reductions in comparison with the coating with chitosan only. However, chitosan coatings with either 0.5 or 1.0% LAE resulted in greater microbial reductions than chitosan coatings with 60 AIT/ml (Fig. 2), while the chitosan coating with 1.0% LAE (CHI1LAE) reduced *Salmonella* more than the same coating supplemented with only 20 µl of AIT per ml and 25 mg of nisin per ml (CHI20AIT25nisin) (Fig. 1).

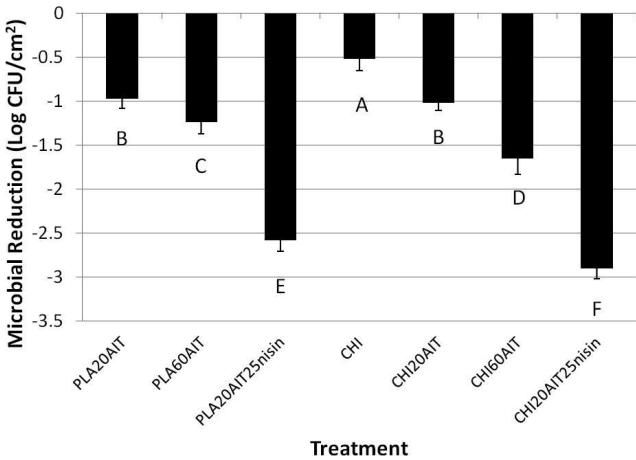


FIGURE 1. Reduction of *Salmonella Enteritidis* on egg shells after antimicrobial coatings. PLA20AIT, polylactic acid (PLA) coating with 20 μ l of AIT per ml; PLA60AIT, PLA coating with 60 μ l of AIT per ml; PLA20AIT25nisin, PLA coating with 20 μ l of AIT per ml and 25 mg of nisin per ml; CHI, chitosan coating; CHI20AIT, chitosan coating with 20 μ l of AIT per ml; CHI60AIT, chitosan coating with 60 μ l of AIT per ml; CHI20AIT25nisin, chitosan coating with 20 μ l of AIT per ml and 25 mg of nisin per ml. Error bars represent the standard deviations of the means. Data with a common letter are not significantly different ($P > 0.05$).

Based on these results, only chitosan coatings containing 1.0 and 0.5% LAE were used in subsequent storage tests.

Effects of egg coatings containing LAE on survival of *Salmonella* during storage. Figure 3 shows the survival of *Salmonella* on egg shells after coating treatments and during storage at 7°C. Coatings containing 1.0% LAE reduced *Salmonella* populations from 6.8 to 1.2 log units at day 1 and then to undetectable levels (<0.5 log CFU/cm²) at day 3. The coating with 0.5% LAE reduced populations to 3.9 log CFU/cm² at day 1, 3.3 log CFU/cm² at day 3, 2.2

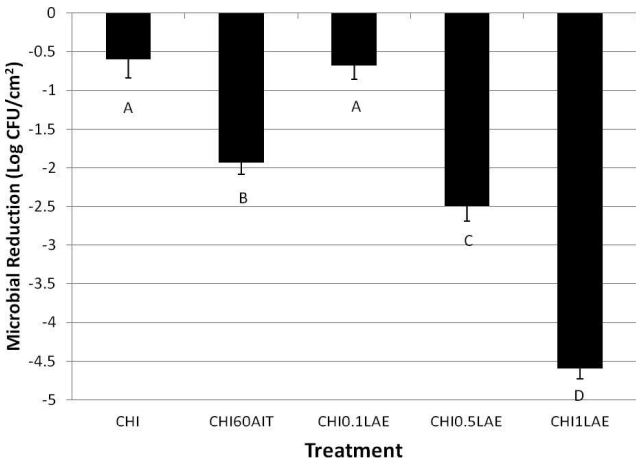


FIGURE 2. Reduction of *Salmonella Enteritidis* on egg shells after antimicrobial coatings. CHI, chitosan coating; CHI60AIT, chitosan coating with 60 μ l of AIT per ml; CHI0.1LAE, chitosan coating with 1.0% LAE; CHI0.5LAE, chitosan with 0.5% LAE; CHI1LAE, chitosan with 1.0% LAE. Error bars represent the standard deviations of the means. Data with a common letter are not significantly different ($P > 0.05$).

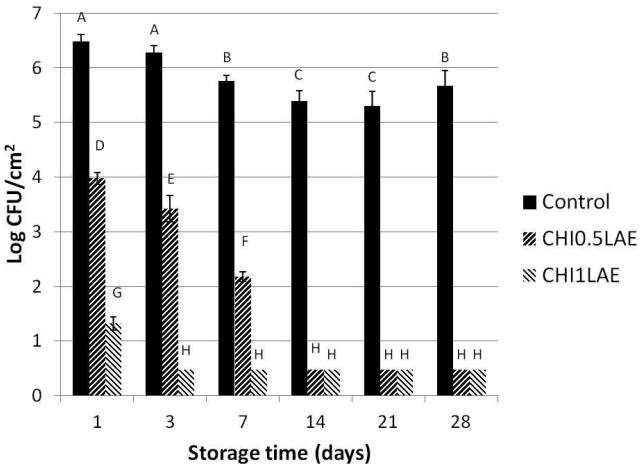


FIGURE 3. Survival of *Salmonella Enteritidis* on egg shells after antimicrobial coatings during storage at 7°C. CHI0.5LAE, chitosan with 0.5% lauric arginate ester (LAE); CHI1LAE, chitosan with 1.0% LAE. Error bars represent the standard deviations of the means. Data with a common letter are not significantly different ($P > 0.05$).

log CFU/cm² at day 7, and finally to undetectable levels at day 14. No regrowth was observed after day 3 (1.0% LAE) or day 7 (0.5% LAE) through the 28 days' storage at 7°C.

Effect of egg coatings containing LAE on weight loss of shell eggs during storage. Weight loss was observed for all eggs during storage at either 7 or 4°C. Shell eggs without coatings (controls) lost ca. 6% of their weight at 98 days at 7°C, while coated eggs lost only ca. 4% (Fig. 4A); nevertheless, there were no significant differences among the three coating treatments. When other coatings were used and stored at a lower temperature (i.e., 4°C) for 110 days, control samples lost 14.1% in weight, while eggs coated with chitosan, PLA plus AIT, and PLA plus AIT plus nisin lost 6.5, 4.3, and 7.5% in weight, respectively, at the end of the storage period. Upon statistical analysis, it was determined that all the coated eggs had significantly less weight loss than did the uncoated eggs (controls).

DISCUSSION

Salmonella is known to contaminate the internal contents of shell eggs via transovarian, transoviductal, or trans-shell transmission. Trans-shell microbial contamination of shell eggs is one of the major *Salmonella* contamination pathways. Some believe that its incidence rate may be significantly higher than that of interior contamination of eggs (4, 40). It is hypothesized that if intrinsic *Salmonella* contamination is reduced or eliminated via better vaccination, quality assurance, and husbandry, treatments may need to be applied only to the exterior of the egg. In such a case, surface decontamination would be a critical step in reducing foodborne illnesses. A commercial recall of hard-cooked eggs occurred in February 2012 due to potential foodborne pathogen contamination. In this incident, the likely source of the contamination was identified as a specific repair project that took place in the egg packaging room (52). This situation suggests that both decontamina-

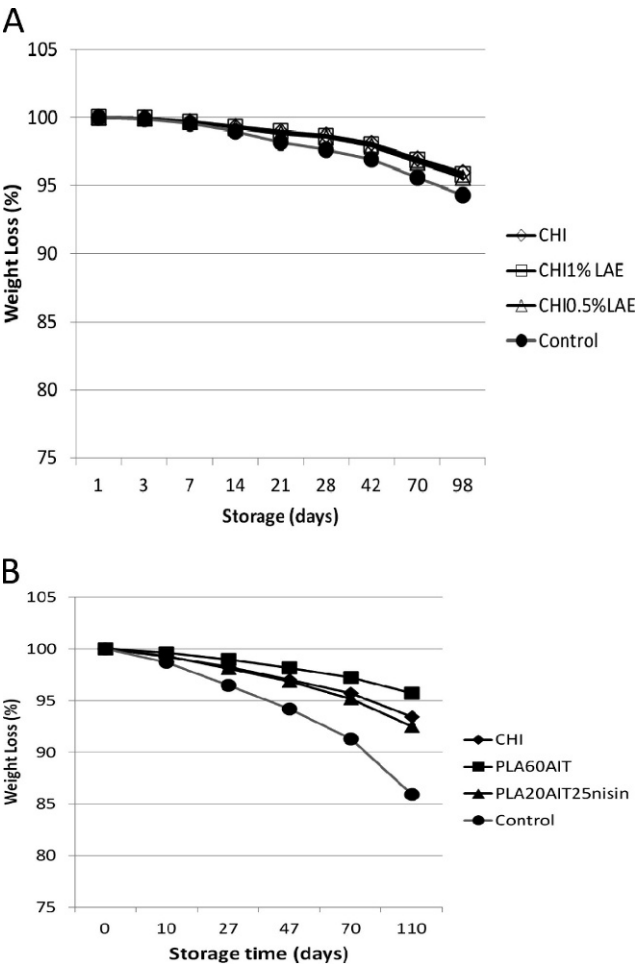


FIGURE 4. Weight loss of shell eggs during storage at 7°C (A) and 4°C (B). CHI0.5LAE, chitosan with 0.5% LAE; CHI1LAE, chitosan with 1.0% LAE. PLA60AIT, PLA coating with 60 μ l of AIT per ml; PLA20AIT25nisin, PLA coating with 20 μ l of AIT per ml and 25 mg of nisin per ml.

tion of eggs and the prevention of recontamination after egg processing (washing, thermal treatment, etc.) are very important to avoid product recalls and outbreaks. Antimicrobial coatings can be applied as a thin film on the eggshell, which reduces or inhibits the growth of *Salmonella* and also provides a protective barrier to prevent recontamination of eggs during transportation, storage, and retail. In our previous study (11) with cantaloupe, antimicrobial coatings not only reduced pathogens already present on the surface but also prevented the attachment and growth of pathogens on coated surfaces. In the present study, the antimicrobial coatings reduced the pathogens to undetectable levels, achieving more than 5 log CFU of inactivation. Antimicrobial coatings, therefore, could play a major role in significantly enhancing the safety of shell eggs and reduce the chances of cross-contamination from the coating process until time of consumption.

Each eggshell contains up to 17,000 pores that serve not only as potential pathways for pathogens and other microorganisms to access the interior of eggs but also as a conduit for movement of carbon dioxide and moisture through the shell. In addition to the antimicrobial efficacy they afford, surface coatings with polymers also reduce CO₂

and moisture loss. In the present study, coated eggs showed significantly less weight loss than uncoated control eggs. Rocculi et al. (44) reported that nonpacked eggs (control) lost about 6.5% of their weight at the end of a 28-day storage period, while all packed samples lost only about 0.5% of their initial weight. Li et al. (37) showed that nonpacked eggs stored for 28 days at 25°C lost 10% of their weight, and Wong et al. (54) reported that weight losses of uncoated and mineral oil-coated eggs were 11.0 and 9.2%, respectively, after 28 days of storage at 4°C. Differences in weight loss between studies may be due to storage conditions, temperature, egg size, hen age, and shell porosity (5, 7). The weight loss of eggs during storage is due mainly to evaporation of water and loss of CO₂, known to decrease internal egg quality by albumen liquefaction and reduction of Haugh units. Consequently, egg pH increases over time, resulting in watery albumen due to the loss of the molecular structure of thick albumen protein as well as in a shift in appearance from transparent to translucent to cloudy when compared with fresh eggs (36). Therefore, weight loss is an index for egg quality degradation, and prevention of weight loss is important for maintaining egg quality.

The addition of shell surface coatings may increase shell strength and potentially decrease the number of cracked eggs. Although we did not conduct shell strength experiments in the present study, numerous studies have documented increased eggshell strength and longer shelf life associated with surface-coated eggs (7–9, 19, 41, 53). In those studies, the food grade egg shell coatings evaluated were composed of mineral oil, WPI, chitosan, shellac, soy protein isolate, wheat gluten, corn zein, and casein. Future studies may address the effect of shell surface coatings on shell strength and other egg quality factors (e.g., Haugh units, albumen and yolk pH, CO₂ loss).

Incorporating antimicrobials into polymers allows the gradual diffusion of target bactericidal or bacteriostatic compounds into food surfaces, hence enhancing antimicrobial efficacy. Chitosan and PLA are biopolymers and possess film-forming properties for use as films or coatings. Chitosan can be used as an edible polymer, while PLA can be used for a food contact polymer (13). AIT, nisin, and LAE are natural antimicrobials and GRAS food additives for their intended purposes as established by the U.S. Food and Drug Administration (3, 14, 25, 34). Based on our findings, AIT, nisin, and LAE alone or in combination might be suitable alternatives to be considered for use by the egg industry, depending on economics and regulatory approval. This study also provides an option for the use of edible or nonedible polymers in food surface coatings. In the case of shell eggs, nonedible (PLA) coatings may be more suitable, since egg shells are typically considered nonedible. However, chitosan itself possesses antimicrobial properties. Additionally, the acid and water solubility of chitosan may have advantages over PLA, which requires solvents to facilitate application. Either way, the antimicrobial coatings developed from this study are applicable for all sizes of farms, egg producers and distributors, which could enhance the microbial safety of the final products and avoid economic losses due to product recalls and human illnesses.

Eggs are an important agricultural commodity, generating millions of dollars in revenue. Since recontamination of egg shells can occur following egg processing, implementation of a technology that not only decontaminates eggs but also prevents further recontamination could be a vital component in mitigating eggborne outbreaks of salmonellosis. In this study, antimicrobial film coatings reduced *Salmonella* more than 5 log CFU/cm² on egg shells and reduced the weight loss of eggs during storage, which provides a simple and economic means of reducing microbial contamination of shell eggs, preventing potential recontamination, preserving egg quality, and mitigating weight loss. The surface-coating treatments could be used in addition to those aimed at preventing transovarian contamination.

The developed coating formula and methods can be used for small- and large-scale egg producers to provide safer eggs and egg products to consumers. It is hoped that results from the present study will lead to new and effective intervention strategies that reduce the risk of foodborne outbreaks due to pathogen contamination. A scaled-up study with quality evaluation will be needed and is recommended prior to commercialization of this technology.

ACKNOWLEDGMENTS

This study was funded by U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) Current Research Information System project no. 1935-41000-092-00D through ARS National Program 108. The authors gratefully acknowledge the technical support of Ms. Anita Parameswaran.

REFERENCES

1. American Egg Board. 2011. Egg industry facts. Available at: <http://www.aeb.org/Retailers/industry.html>. Accessed September 2011.
2. Anonymous. 1993. Advisory Committee on the Microbiological Safety of Food. Report on *Salmonella* in eggs, p. 26–27. Available at: <http://www.food.gov.uk/multimedia/pdfs/acmsfosalmonellaineggs.pdf>. Accessed October 2012.
3. Bakal, G., and A. Diaz. 2005. The lowdown on lauric arginate. *Food Qual. Mag.* 2/3:60–61.
4. Barrow, P. A., and M. A. Lovell. 1991. Experimental infection of egg-laying hens with *Salmonella enteritidis* phage type 4. *Avian Pathol.* 20:335–348.
5. Bhale, S., H. K. No, W. Prinyawiwatukul, A. J. Farr, K. Nadarajah, and S. P. Meyers. 2003. Chitosan coating improves shelf life of eggs. *J. Food Sci.* 68:2378–2383.
6. Burow, H. 1992. Dominanz von *Salmonella enteritidis* bei Isolierungen aus Lebensmittel tierischer Herkunft in Nordbayern. *Fleischwirtschaft* 72:1045–1050.
7. Caner, C. 2005. The effect of edible eggshell coatings on egg quality and consumer perception. *J. Sci. Food Agric.* 85:1897–1902.
8. Caner, C. 2005. Whey protein isolate coating and concentration effects on egg shelf-life. *J. Sci. Food Agric.* 85:2143–2148.
9. Caner, C., and O. Cansiz. 2008. Chitosan coating minimizes eggshell breakage and improves egg quality. *J. Sci. Food Agric.* 88:56–61.
10. Centers for Disease Control and Prevention. 2011. Investigation update: multistate outbreak of human *Salmonella* Enteritidis infections associated with shell eggs. Available at: <http://www.cdc.gov/salmonella/enteritidis>. Accessed September 2011.
11. Chen, W., T. Z. Jin, J. B. Gurtler, D. J. Geveke, and X. Fan. 2012. Inactivation of *Salmonella* on whole cantaloupe by application of an antimicrobial coating containing chitosan and allyl isothiocyanate. *Int. J. Food Microbiol.* 155:165–170.
12. Cho, J. M., S. K. Park, Y. S. Lee, and C. O. Rhee. 2002. Effects of soy protein isolate coating on egg breakage and quality of eggs during storage. *Food Sci. Biotechnol.* 11:392–396.
13. Conn, R. E., J. J. Kolstad, J. F. Borzelleca, D. S. Dixler, L. J. Filer, Jr., B. N. Ladu, Jr., and M. W. Pariza. 1995. Safety assessment of polylactide (PLA) for use as a food-contact polymer. *Food Chem. Toxicol.* 33:273–283.
14. Delaquis, P. J., and G. Mazza. 1995. Antimicrobial properties of isothiocyanates in food preservation. *Food Technol.* 49(11):73–74, 79–84.
15. European Food Safety Authority. 2005. Opinion of the scientific panel on biological hazards on the request from the commission related to the microbiological risks on washing of table eggs. *EFSA J.* 269:1–39.
16. European Food Safety Authority. 2007. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. *EFSA J.* 130: 34–117.
17. European Food Safety Authority. 2009. The community summary report on food-borne outbreaks in the European Union in 2007. *EFSA J.* 271:1–102.
18. Gurtler, J. B., R. B. Rivera, H. Q. Zhang, and C. H. Sommers. 2010. Behavior of avirulent *Yersinia pestis* in liquid whole egg as affected by antimicrobials and thermal pasteurization. *J. Food Saf.* 30:537–557.
19. Heath, J. L. 1977. Chemical and related changes in egg albumen during storage. *Poult. Sci.* 56:822–828.
20. Herald, T. J., R. Gnanasambandam, B. H. McGuire, and K. A. Hachmeister. 1995. Degradable wheat gluten films: preparation, properties and applications. *J. Food Sci.* 60:1147–1150, 1156.
21. Holt, P. S., R. H. Davies, J. Dewulf, R. K. Gast, J. K. Huwe, D. R. Jones, D. Waltman, and K. R. Willian. 2011. The impact of different housing systems on egg safety and quality. *Poult. Sci.* 90:251–262.
22. Hou, H., R. K. Singh, P. M. Muriana, and W. J. Stadelman. 1996. Pasteurisation of intact shell eggs. *Food Microbiol.* 13:93–101.
23. Humphrey, T. J., A. Whitehead, A. H. L. Gawler, A. Henley, and B. Rowe. 1991. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens eggs. *Epidemiol. Infect.* 106:489–496.
24. International Commission on Microbiological Specifications for Foods. 1998. Eggs and egg products, p. 475–520. In T. A. Roberts, J. I. Pitt, J. Farkas, and F. H. Grau (ed.), *Microorganisms in foods 6*. Blackie Academic and Professional, London.
25. Isshiki, K., K. Tokuoka, R. Mori, and S. Shiba. 1992. Preliminary examination of allyl isothiocyanate vapor for food preservation. *Biosci. Biotechnol. Biochem.* 56:1476–1477.
26. Jin, T. 2010. Inactivation of *Listeria monocytogenes* in skim milk and liquid egg white by antimicrobial bottle coating with polylactic acid and nisin. *J. Food Sci.* 75(2):M83–M88.
27. Jin, T., and J. B. Gurtler. 2011. Inactivation of *Salmonella* in liquid egg albumen by antimicrobial bottle coatings infused with allyl isothiocyanate, nisin and zinc oxide nanoparticles. *J. Appl. Microbiol.* 110:704–712.
28. Jin, T., and J. B. Gurtler. 2012. Inactivation of *Salmonella* on tomato stem scars by edible chitosan and organic acid coatings. *J. Food Prot.* 75:1368–1372.
29. Jin, T., and B. Niemira. 2011. Application of polylactic acid coating with antimicrobials in reduction of *Escherichia coli* O157:H7 and *Salmonella* Stanley on apples. *J. Food Sci.* 76(3):M184–M188.
30. Jin, T., and H. Zhang. 2008. Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging. *J. Food Sci.* 73(3): M127–M134.
31. Jones, D. R., and M. T. Musgrove. 2005. Effects of extended storage on egg quality factors. *Poult. Sci.* 84:1774–1777.
32. Jones, D. R., J. B. Tharrington, P. A. Curtis, K. E. Anderson, K. M. Keene, and F. T. Jones. 2002. Effects of cryogenic cooling of shell eggs on egg quality. *Poult. Sci.* 81:727–733.
33. Kapperud, G., S. Gustavsen, I. Hellesnes, A. H. Hansen, J. Lassen, J. Him, M. Jahkola, M. A. Montenegro, and R. Helmuth. 1990. Outbreak of *Salmonella typhimurium* infection trace to contaminated

- chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. *J. Clin. Microbiol.* 28:2597–2601.
34. Kim, Y. S., E. S. Ahn, and D. H. Shin. 2002. Extension of shelf life by treatment with allyl isothiocyanate in combination with acetic acid on cooked rice. *J. Food Sci.* 67:274–279.
35. Knight, D. W., M. Bowrey, and D. J. Cooke. 1972. Preservation of internal egg quality using silicone fluids. *Br. Poult. Sci.* 13:587–593.
36. Lee, S. H., H. K. No, and Y. H. Jeong. 1996. Effect of chitosan coating on quality of egg during storage. *J. Korean Soc. Food Sci. Nutr.* 25:288–293.
37. Li, L. Y., C. C. Lai, and S. G. Gilbert. 1985. Keeping quality of eggs packaged in acrylonitrile pouches. *J. Food Proc. Preserv.* 9:179–187.
38. Liu, L. S., T. Jin, D. Coffin, and K. Hicks. 2009. Preparation of antimicrobial membranes: coextrusion of poly(lactic acid) and Nisaplin in the presence of plasticizers. *J. Agric. Food Chem.* 57: 8392–8398.
39. Liu, L. S., T. Jin, D. R. Coffin, C. K. Liu, and K. Hick. 2010. Poly(lactic acid) membranes containing bacteriocins and EDTA for inhibition of the surface growth of gram-negative bacteria. *J. Appl. Polym. Sci.* 117:486–492.
40. Luber, P. 2009. Cross-contamination versus undercooking of poultry meat or eggs—which risks need to be managed first? *Int. J. Food Microbiol.* 134:21–28.
41. Meyer, R., and J. V. Spencer. 1973. The effect of various coatings on shell strength and egg quality. *Poult. Sci.* 52:703–711.
42. Obanu, Z. A., and A. A. Mpiere. 1984. Efficiency of dietary vegetable oils in preserving the quality of shell eggs under ambient tropical conditions. *J. Agric. Food Chem.* 35:1311–1317.
43. Public Health Agency of Canada. 2003. Material safety data sheets. Available at: <http://www.phac-aspc.gc.ca/msds-ftss/msds135e-eng.php>. Accessed September 2011.
44. Rocculi, P., E. Cocci, F. Sirri, S. Romani, and A. Meluzzi. 2009. MAP storage of shell hen eggs. Part 1. Effect on physico-chemical characteristics of the fresh product. *LWT Food Sci. Technol.* 42:758–762.
45. Schroeder, C. M., A. L. Naugle, W. D. Schlosser, A. T. Hogue, F. J. Angulo, J. S. Rose, E. D. Ebal, W. T. Disney, K. G. Holt, and D. P. Goldman. 2005. Estimate of illnesses from *Salmonella* enteritidis in eggs, United States, 2000. *Emerg. Infect. Dis.* 11(1):113–115.
46. Schuman, J. D., B. W. Sheldon, J. M. Vandepopuliere, and H. R. Ball, Jr. 1997. Immersion heat treatments for inactivation of *Salmonella* enteritidis with intact eggs. *J. Appl. Microbiol.* 83:438–444.
47. Smith, R. 25 July 2011. Food ‘stars’ on the rise. *Feedstuffs*. Available at: http://fdsmagissues.feedstuffs.com/fds/PastIssues/FDS8330/fds06_8330.pdf. Accessed September 2012.
48. St. Louis, M. E., D. L. Morse, M. E. Potter, T. M. DeMelfi, J. J. Guzewich, R. V. Tauxe, P. A. Blake, and the *Salmonella enteritidis* Working Group. 1988. The emergence of grade A eggs as a major source of *Salmonella* enteritidis infections: new implications for the control of salmonellosis. *JAMA (J. Am. Med. Assoc.)* 259:2103–2107.
49. Telo, A., B. Bijó, K. Sulaj, and E. Beli. 1999. Occurrence of *Salmonella* spp. into Albania. *Int. J. Food Microbiol.* 49:169–171.
50. U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Pasteurization of liquid eggs. 9 CFR Part 590.570. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC.
51. U.S. Department of Agriculture, Food Safety and Inspection Service. 2005. Shell eggs and egg products: risk assessments for *Salmonella* Enteritidis in shell eggs and *Salmonella* spp. in egg products (report). Available at: http://origin-www.fsis.usda.gov/Science/Risk_Assessments/index.asp. Accessed September 2011.
52. U.S. Food and Drug Administration. 2012. Recall—firm press release. Available at: <http://www.fda.gov/Safety/Recalls/ucm289920.htm>. Accessed September 2012.
53. Vought, K. J., and S. R. Tatini. 1998. *Salmonella* enteritidis contamination of ice cream associated with a 1994 multistate outbreak. *J. Food Prot.* 61:5–10.
54. Wong, Y. C., T. J. Herald, and K. A. Hachmeister. 1996. Evaluation of mechanical and barrier properties of protein coatings on shell eggs. *Poult. Sci.* 75:417–422.
55. Xie, L., N. S. Hettiarachchy, Z. Y. Ju, J. Meullenet, H. Wang, M. F. Slavik, and M. E. Janes. 2002. Edible film coating to minimize eggshell breakage and reduce post-wash bacterial contamination measured by dye penetration in eggs. *J. Food Sci.* 67:280–284.